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EVALUATION OF SURFACE pH AS A FRESHNESS INDEX FOR FISH FILLETS 1/

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At the present time organoleptic observations are the only generally accepted criteria for judging the freshness of fish. Such tests are very inaccurate, but no accurate objective test has yet been devised which meets the requirements of simplicity, rapidity, low cost, and also does not mutilate the sample and thus render it unfit for sale.

If the pH of fish flesh could be correlated with fish freshness, such a test would come very close to meeting the requirements of an ideal test. pH determinations employing a pH meter are simple, rapid, and accurate, yet the samples are not destroyed. Substitution of pH test papers for the pH meter would increase the simplicity and economy of such a test.

Wood, Sigurdsson, and Dyer (1942) suggested that tests on the surface of fish flesh are much more delicate than those on composite samples containing both surface and interior flesh. This is due to the fact that spoilage is much more rapid at the surface than in the interior of fillets. A more recent paper by the same authors, Dyer, Sigurdsson, and Wood (1944), suggests the use of pH on the surface of fillets as an index of freshness. These authors indicate that this is a reliable test even though the pH values of the interior, or of the composite samples, are not sensitive indicators of fish freshness.

The U. S. Army Quartermaster Corps buys fish following an inspection by the Army Veterinary Corps. All tests employed by the Veterinary Corps are organoleptic, and consequently the quality of the fish purchased depends entirely on the judgment of the individual inspector. For this reason the U. S. Army Quartermaster Corps expressed the need for an objective test to replace, or at least to supplement, the organoleptic examination and asked the U. S. Fish and Wildlife Service to investigate the test proposed by Dyer et al (1944). This investigation was then undertaken with the following objects in view:

1. To study the surface pH test to determine whether or not it would of of value to the Army Veterinary Corps inspectors as a freshness yardstick for fish fillet purchases.
2. To simplify it further.
3. To apply the test to the types of fish the U. S. Army generally purchases for mess purposes. These included various species not previously tested by Dyer et al. (1944).

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The studies here reported were made during the months of September, October, and November, 1944, in laboratory space furnished to the U. S. Fish and Wildlife Service by the Gorton-Pew Fisheries Company, Limited, at Gloucester, Massachusetts.

Experimental Procedure

Handling Fish Samples: Fish were received directly from the commercial fishing boats in as fresh a state as possible. Gillnet boats were out for about 10-hour periods; consequently, those fish described as "gillnet"-caught were only six to seven hours out of the water when purchased. Otter trawlers were out for periods of one to two weeks. The fish described as "otter trawl"-caught were obtained from the top of the load wherever possible and, therefore, were still fresh when received. During the storage period on otter-trawl boats, the fish had been iced according to commercial practice.

The fish to be tested (haddock, whiting, dabs, pollock, cod, rosefish, and gray sole) were filleted immediately upon their receipt at the dock. As the fillets from each fish left the filleter's knife, they were paired and wrapped together in a piece of parchment or waxed paper and stored in crushed ice. At all times during the storage period, an excess of ice covered them, so that their temperature was kept constant. At intervals of one or two days, until the fish were completely spoiled, the fillets from six[1] fish were removed from the ice and one fillet from each pair was examined, while its duplicate was wrapped in double parchment or waxed paper and then in plain wrapping paper and placed in a cold room held at -15 to -18°C.(5 to -0.4°F.).

Duplicate fillets were frozen because any test, to be practical for Army use, had to be applicable to fish which had been frozen since most of the fish used by the Army is frozen immediately after filleting and critical inspections are made on the thawed product. These frozen duplicates were held from two weeks to two months at -15 to -18°C. until time was available for their examination. Because the investigation was necessarily of short duration, it was impossible to determine the effect of prolonged frozen storage.

Laboratory Technique: In the laboratory the fillets were tested using a pH meter.[2] Careful checks were made on the meter with commercially prepared buffer solutions at pH 7 at the beginning of each day's work and about every two hours during the examinations.

Preliminary studies indicated that washing electrodes between fillets was unnecessary because the effect of residual films on the glass electrode was overcome by each new contact with the fillet surface. It was found that the effect of fork holes on the pH was not noticeable unless the portion around the hole was very badly discolored and decomposed. The effect of temperature variation on the readings was found to be unimportant for the temperature range in which the fillets were examined, provided the temperature compensator on the pH meter was used.

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[1] Fitzgerald and Conway (1937) found that a minimum of five fish was required to represent the quality of a batch of 2,000 pounds of haddock.

[2] This was a Beckman Model G with external glass (Beckman No. 1190) and calomel (Beckman No. 1170) electrodes.

Preliminary to the examination, the fillets were placed on a table in a room at 20 to 30°C. (68 to 86°F.) and allowed to remain until their temperature had changed from that of melting ice to 10 to 15°C.(50 to 59°F.). Both unfrozen[3] and frozen fish were treated in this manner. Though the frozen fish took three to four hours to thaw, it was believed inadvisable to apply heat or water.

The pH values of the surface and freshly cut interior portions were made at points designated (Fig. 1). The fillet was held firmly against the rigidly supported electrodes, but not firmly enough to puncture the flesh, and a few seconds were allowed for equilibrium to be attained before the reading was taken.

The odor of the raw, uncooked fillet was noted when the readings for both the surface and the interior had been obtained. At the beginning of the investigation the opinions of three or four people were averaged, while during the latter part of the study only the author's opinion was considered. Since his opinions had always been in agreement with the average of the other participants, this was believed acceptable.

It may be noted in the accompanying graphs and tables that the organoleptic data were classified as fresh, flat, sweet, stale, and putrid. A fillet designated as "fresh" had the normal odor of freshly caught fish. If it was "flat" there was an absence of odor--normal or otherwise. A "sweet" fillet had an odor not especially unpleasant but reminiscent of watermelon. A "stale" fillet had a characteristic ammonia-like odor (odor of ammonia and other mixed amines) but had not reached the "putrid" stage at which point the odor became obnoxious (hydrogen sulfide, indole, skatole, etc. were present). All fish examined in this study spoiled in the above manner except whiting and rosefish. At the sweet stage whiting developed a perfume-like aroma instead of the usual watermelon-like odor. Rosefish did not become sweet but passed directly from flat to slightly stale.

The author and those who co-operated with him considered the fillets edible through the "sweet" stage, of questionable edibility at "very sweet" and "slightly stale," and inedible at "stale" and at more advanced stages of decomposition. This designation of the exact point at which the fish became inedible was determined by judges in the habit of eating fish only of the very freshest nature. Less discriminating people might have placed such a point elsewhere.

Interpretation of Data

Accuracy of Methods: A study of values obtained at the various surface sampling points shown (Fig. 1) revealed that the fiducial limits[4] of the seven surface pH readings on individual fillets was, on all samples except those in advanced stages of decomposition, always as small as or smaller than ± 0.18. The fiducial limits of the mean[4] were as small as or smaller than ± 0.08, except in the most advanced stages of spoilage (Table 1). These values were believed to

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[4] The "fiducial limits" give the limits within which 95 per cent of the values obtained will be expected to fall. In this case (seven samples), the standard deviation multiplied by $2\frac{1}{2}$ gave the "fiducial limits," and the standard deviation of the mean multiplied by $2\frac{1}{2}$ gave the "fiducial limits of the mean."

[3] "Unfrozen" is used instead of "fresh" to avoid confusion between the use of the word "fresh" to signify lack of freezing and "fresh" as an organoleptic criterion.

be sufficiently small for the required accuracy of the test. In the data presented here, all surface pH averages may be assumed to be within ± 0.08 pH of the true mean for the fillet described, unless that fillet is "very stale" or worse.

Table 1

Precision of pH Determination on Haddock and Gray Sole

Odor Rating	Expected variation among individual surface pH determinations on fillets at various degrees of spoilage[1]					
	Fiducial limits (standard deviation X 2½)			Fiducial limits of the mean (standard deviation of mean X 2½)		
	Unfrozen otter-trawl haddock	Frozen otter-trawl haddock	Unfrozen otter-trawl gray sole	Unfrozen otter-trawl haddock	Frozen otter-trawl haddock	Unfroz otter-tr gray so
Fresh.....	.10	.06	.18	.04	.02	.07
Flat.....	.11	.07	.15	.04	.03	.06
Sl. sweet.....	.16	---	---	.06	---	---
Sweet.....	---	---	.18	---	---	.07
Sl. stale.....	.18	.17	.18	.07	.07	.08
Stale.....	---	.18	.20	---	.07	.08
Very stale.....	.19	.39	---	.07	.15	---
Sl. putrid.....	.70	---	---	.27	---	---
Putrid.....	---	.27	---	---	.10	---

[1] See Fig. 1 for the positions of the seven surface pH readings taken on each fillet.

It must be understood that organoleptic tests are not always accurate. Had a strictly reliable organoleptic method been available, perhaps better correlation with pH would have been obtained in certain instances. Reference to the graphs accompanying this report reveals that when pH is plotted against organoleptic rating (Figs. 3 to 7), the standard deviation is quite large at times. However, when pH is plotted against storage time (Figs. 3 to 7) the standard deviation is less as a general rule. Thus storage time is indicated to be more dependable than organoleptic rating in these instances.

Theory of pH Changes: Figure 2 shows the ideal curve upon which the surface pH concept as described by Dyer, Sigurdsson, and Wood (1944) is based. The flesh of live fish, such as haddock, has a pH value close to neutrality. According to Benson (1928), however, this is true only of rested muscle; the flesh of fish which have struggled on being caught (gillnet, otter-trawl, and line-caught fish) is slightly acid on death, and it will become more acid as the processes of rigor mortis take place. Within four to 24 hours after the death of the fish the pH of the flesh reaches a minimum in the neighborhood of pH 6.5 or lower. In the case of fillets surface and interior pH values are the same at this stage since no surface bacterial action has taken place. For a few days the pH of both the surface and the interior remains in the vicinity of 6.5 The fillets are still considered to be in a fresh condition. After a period of time which varies with the temperature, the species of fish, and many other

factors, bacterial action at the surface of the fillet releases amines, chiefly trimethylamine, which tend to cause a rise in the pH at the surface. Thus the surface pH increases more rapidly than the interior pH, since the bacteria and/or their metabolic end products filter to the interior only very slowly. This differential has been taken by Dyer et al. (1944) as significant in determining the difference between very fresh fish and fish at incipient spoilage, even though the surface pH values may be similar. For example, the surface pH immediately on death (Line A, Fig. 2) may be the same as that when spoilage is under way (Line P, Fig. 2), but the interior pH in the latter case would be much lower.

In view of the importance of such pH differences between the surface and interior, points representing fillets whose interior pH values were close to the average of the seven surface values have been encircled on the graphs accompanying this report, while a cross is used to mark those points representing fillets on which one or more of the interior pH values were significantly lower than this average. If an interior pH departed from the mean of the seven surface values by more than two and a half times the standard deviation of the surface readings of all the fresh fillets, the fact was considered significant. As reference to Figs. 3 to 7 will demonstrate, the greater proportion of encircled pH values occurred on fillets in a comparatively fresh condition; thus the contention advanced by Dyer et al. (1944) that surface pH rises faster than interior pH is substantiated.

Presentation of Data[5] Figures 3 to 7 show the relation of surface pH of fillets of various species to storage time in ice after filleting and to organoleptic rating.

For all species studied it was found that the pH rose as spoilage progressed. With some species the dependability of the correlation of pH with the degree of spoilage was good, but with others it was poor.

It is evident (Fig. 3) that pH 6.7 was the dividing line between fresh fillets of haddock and those at incipient spoilage. These results are in agreement with the work of Dyer et al. (1944). An experiment run with gillnet-caught haddock showed results very close to those obtained with the otter-trawl haddock, but the limiting line between fresh and spoiling fish was pH 6.8 instead of pH 6.7. This difference might very well have been experimental error.

The correlation of pH with freshness of whiting (Fig. 4) was nearly as good as that for haddock. With this species, however, the pH range of the fresh fillets (pH 6.9 to 7.3) was much higher than that for haddock. Dabs (Hippoglossoides platessoides) fell into the same category as whiting, though the pH range of fresh samples (pH 6.7 to 7.0) was lower than that for whiting. No data are presented for this species.

With haddock, whiting, and dabs the pH of an individual fillet gave some idea of its condition as evidenced by the consistent rise in pH as spoil-

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[5] Because approximately 10,000 pH determinations were made during the course of the investigation, all of this material could not possibly be presented here for lack of space. For this reason, no data are presented for the frozen duplicate fillets, and graphs for some species of fish were deleted because they were very similar to those included with the report.

age progressed and by the absence of any serious overlap of individual points at different levels. With the rest of the species studied, however, the surface pH values of individual fillets lost their significance and only average pH values of several fillets of equal quality could be relied on to give a correlation with storage time or organoleptic rating. In some cases even this average was very erratic. If more than six fillets had been examined each day, smoother curves would have resulted.

The data for pollock are presented (Fig. 5); the pH range for the fresh fillets was very wide (from about 6.5 to 7.1). All but a few of the fillets whose interior pH values were close to the values at the surface (as indicated by encircled points) fell in the "fresh" group, and few were included in this group in which the interior pH was significantly lower than the surface pH. This would indicate, according to the theory of the surface concept, that those fresh fillets whose pH values were quite high might be at the extremely fresh stage (as indicated by Line A, Fig. 2). This idea was not borne out by the data, however. A comparison of the data used in plotting the two curves (Fig. 5) showed that pH readings above 6.9 occurred on fresh fillets on the second, third, and fifth days as well as on the first day.

Dyer et al. (1944) reported that the test worked well for cod. The data for cod (Fig. 6) show that, in these experiments, the pH values of individual fillets of a given age or of a given odor rating had little meaning, while the average of these figures showed an upward trend as spoilage progressed.

The data for gray sole, as presented (Fig. 7), showed that pH may not be a reliable index of the condition of fillets even though a general trend upward is easily seen. In this case the surface pH values of individual fillets had very little meaning, and even the mean of several of equal quality was not a wholly dependable criterion. The data for rosefish (Sebastes marinus) are not presented, but the results when plotted look very much like those of gray sole (Fig. 7); that is, individual points overlapped, the line drawn between mean pH values of fillets of equal quality varied erratically, and the total pH shift was not great.

When the data for the thawed duplicate samples were plotted (graphs not presented), it was found that the pH values as a whole dropped considerably as a result of freezing and/or storage in the frozen condition. Upon careful scrutiny of surface pH averages of individual fillets, however, it was found that this tendency for pH to drop on freezing was not at all consistent, and occasional values even rose after such treatment. Because of such irregularity no correction factor can be assigned to a given species. Therefore, since the changes in pH owing to freezing are often very marked, the application of the test to thawed fish as a freshness index seems impractical.

Simplification of the Test: An attempt was made to simplify the test by the use of pH test papers, but none was found in the proper range which was sufficiently accurate for the purpose. This was not surprising in view of similar results reported by other workers, Kolthoff and Rosenbloom (1937). It is felt, however, that the use of the pH meter is not a serious drawback to the test.

Discussion

In considering the application of the test to commercial practice, certain difficulties arise. First, it gives an indication of spoilage which occurs

only after the filleting process. Undoubtedly, spoilage occurring previous to filleting does have some effect on the test, but surface pH values on new fillets would give no more indication of freshness than those on composite samples or internal flesh, since the filleting operation makes a fresh cut and, therefore, a surface pH taken immediately after filleting would not be a surface test but rather an interior test. If the test could be applied to a whole or eviscerated fish, this difficulty might be avoided; however, in their reference to its use in this manner, Dyer et al. (1944) did not describe how it could be done. The author would expect it to be very difficult to correlate surface pH of whole or eviscerated fish with the freshness of the flesh.

The next limitation, and probably one of equal importance, is the effect of fillet dips on the test. On the Atlantic coast, and to a small extent on the Pacific coast, fillets are dipped in brine after the filleting process. Occasionally alkaline dips are used to enhance the color and, therefore, the salability of the fillet. If surface pH were used as a measure of freshness, any of the dips used at present might affect the test markedly. Also, when the test became well known, it would be an easy matter for the processor to introduce acid products into the dipping solution of fish of questionable quality and thus lower the pH to correspond to values obtained on a strictly fresh product. Development of the test on whole or eviscerated fish would not circumvent this difficulty since the practice of using carbonate solutions to remove and neutralize malodorous materials from whole fish has been well established in some sections.

As stated previously, the test probably applies only to fish which have never been frozen since, on freezing, erratic pH changes occur which are not connected with freshness in any way.

These limitations have been mentioned to show that, even if it were perfected, the test would not be of practical value to the Army. Furthermore, in commercial application its drawbacks, as outlined above, are not to be disregarded. The test has proved of great value from the standpoint of theoretical interest, however, since it has added to our information on fish spoilage. For laboratory research it will prove very useful.

Before any general application could be made, it would be necessary to determine different limits for each species and to determine the number of fillet examinations that would be required to give a true indication of the condition of a lot of fish. Fitzgerald and Conway (1937) stated that five representative fish indicated the quality of a 2,000-pound lot, but this does not mean that the pH values of five fish represent the quality of that lot, since wide pH variations often occur between fish of equal quality (Figs. 5, 6, and 7). It would also be desirable to determine the effect of (1) variation in types of bacteria present, (2) variation in bacterial load, (3) differences in area of catch, (4) differences in method of catch, and (5) differences in season of the year.

Summary

The surface pH test as described by Dyer, Sigurdsson, and Wood (1944) as a method for determining the freshness of fish fillets was studied, using haddock, whiting, dabs, pollock, cod, rosefish, and gray sole.

As determined by comparison of pH changes with organoleptic grading and with time of storage in ice, the surface pH of fillets tended to rise in all

cases as spoilage progressed. The best correlation was obtained with haddock, whiting, and dabs. With the other species examined wide variations occurred in pH among fillets in an equal state of freshness. Thus individual pH values had less significance in the case of these latter species, and only the mean pH of several fillets of equal quality gave a good indication of pH trends on spoilage.

The data obtained on thawed fish did not show a dependable correlation between pH and freshness because of erratic pH changes, probably owing to the freezing and/or storage in the frozen condition.

Even if the pH test were perfected, it would be of limited value because it gives a reliable index of spoilage occurring only after the filleting process and because it would be affected by acid or alkaline dips. These limitations reduce its value for practical field work but do not preclude its use as a laboratory test in research work.

Acknowledgment

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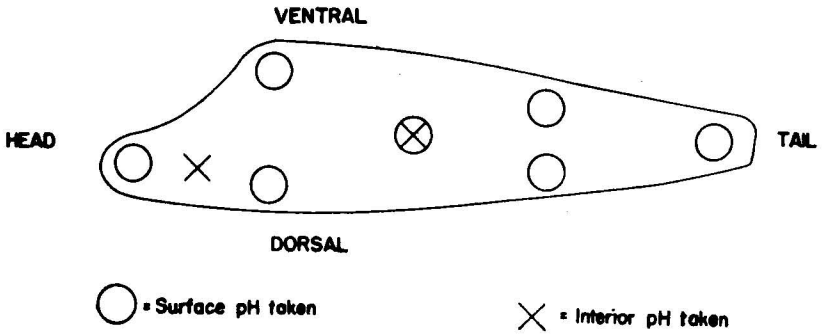

FIG. 1. Positions on fillet at which pH readings were obtained.

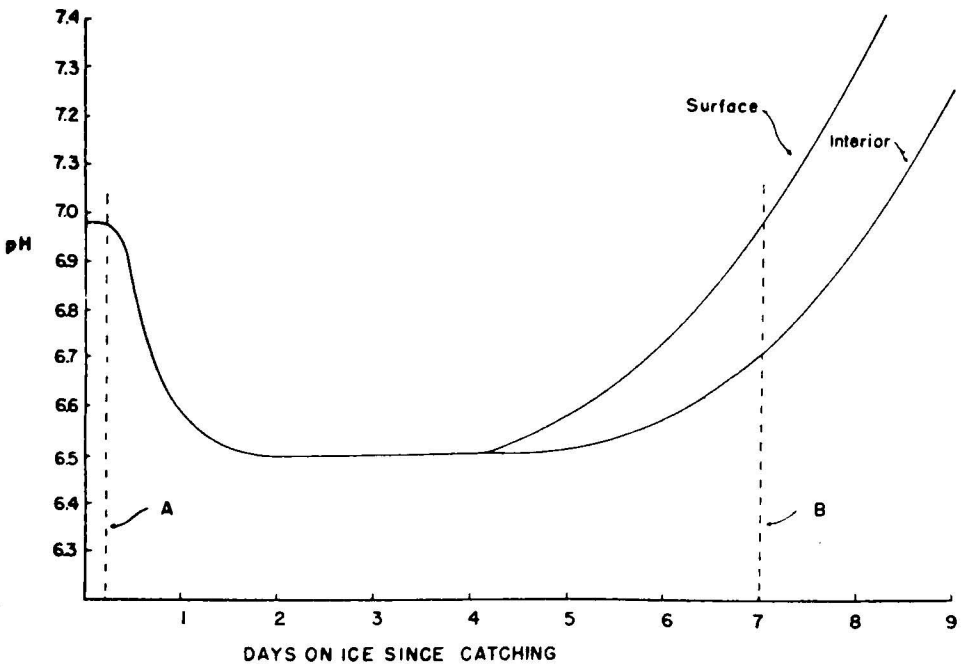

FIG. 2. pH changes occurring during spoilage of such fish as haddock, etc. (This curve is a theoretical ideal and does not represent experimental data.)

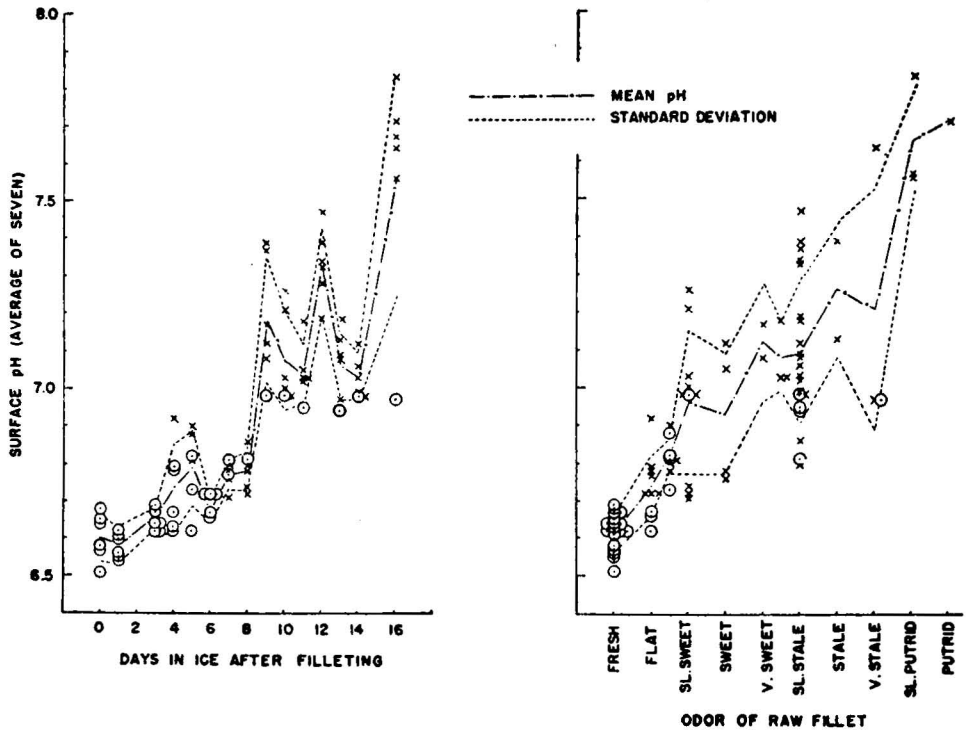

FIG. 3. pH changes during spoilage of otter-trawl haddock (*Melanogrammus aeglefinus*) fillets.[6]

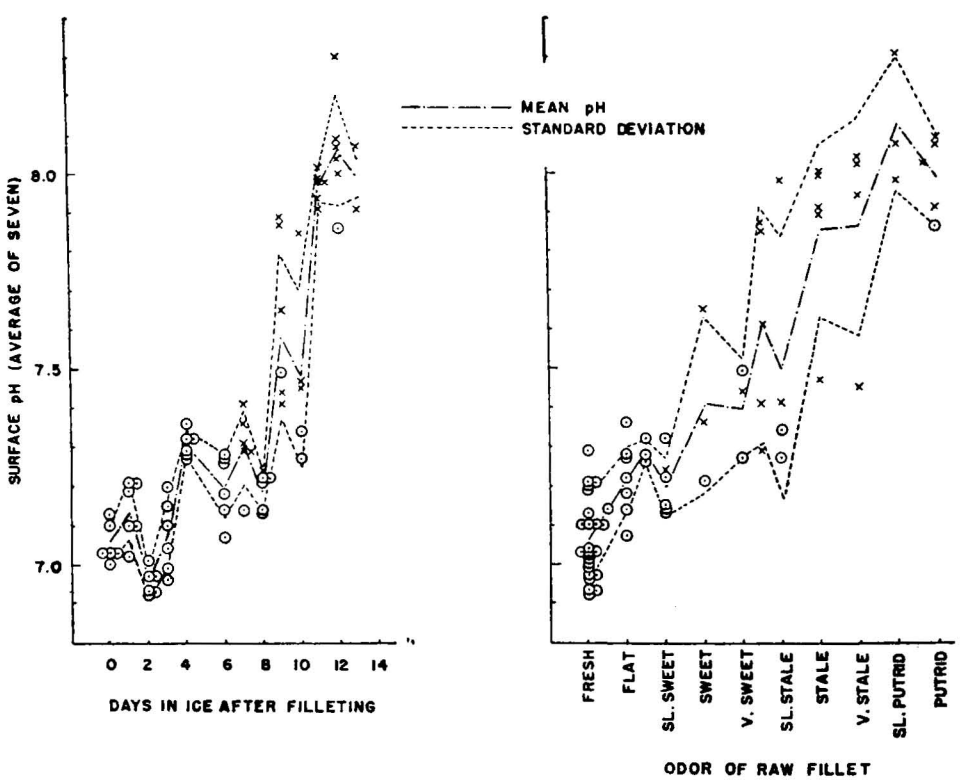

FIG. 4. pH changes during spoilage of whiting (*Merluccius bilinearis*) fillets.[6]

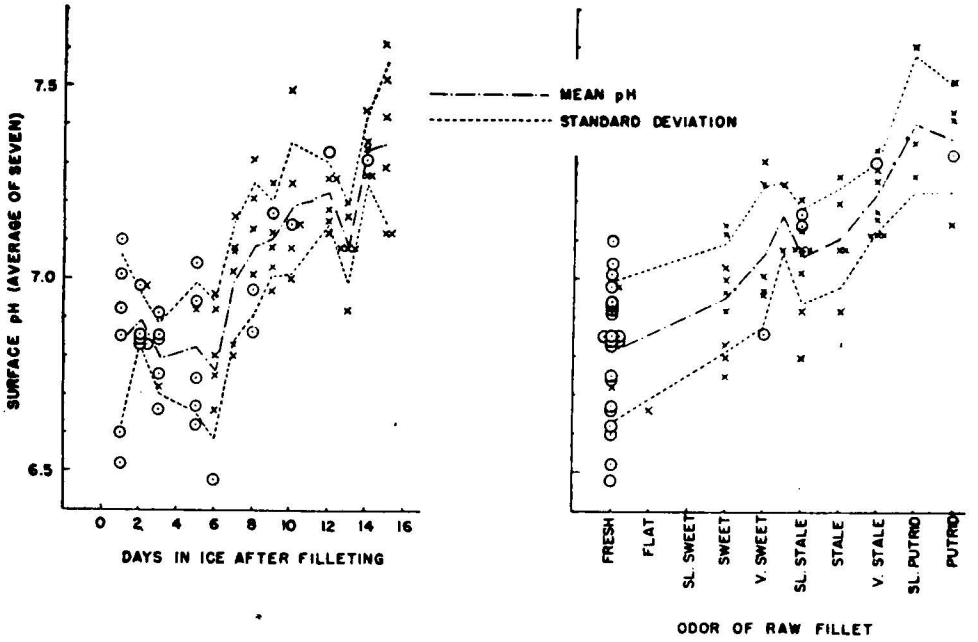

FIG. 5. pH changes during spoilage of gillnet pollock (*Pollachius virens*) fillets.[6]

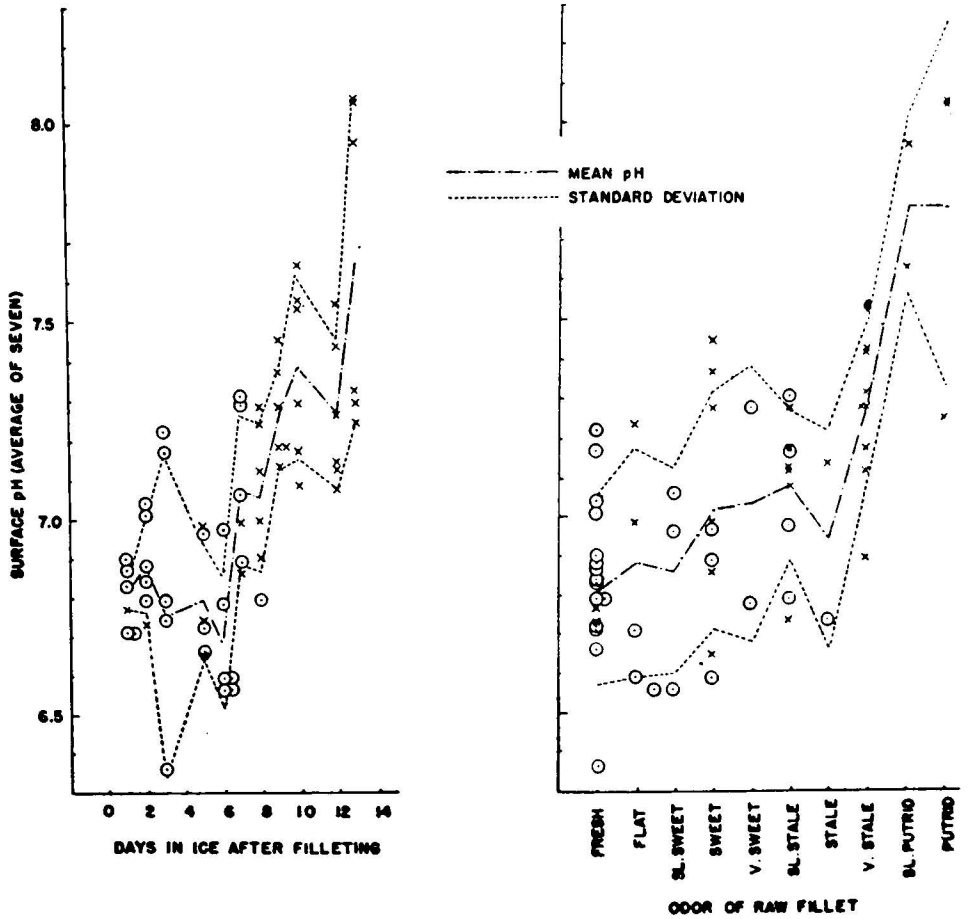

FIG. 6. pH changes during spoilage of gillnet cod (*Gadus callarias*) fillets.[6]

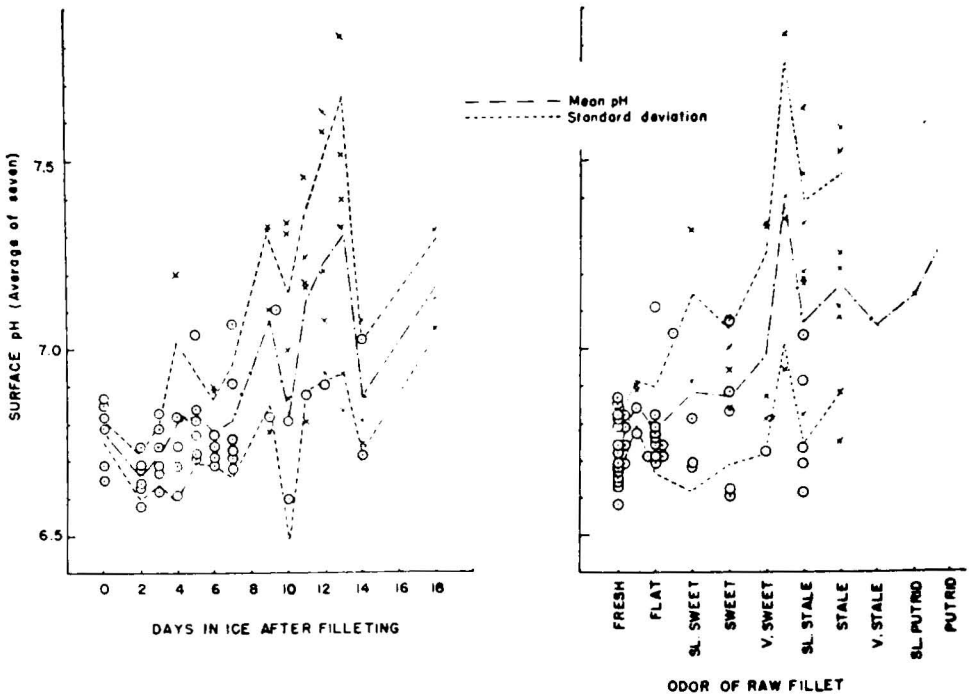

FIG. 7. pH changes during spoilage of otter-trawl gray sole (*Glyptocephalus cynoglossus*) fillets.[6]

[6] In Figs. 3 to 7, encircled points indicate that both interior pH values taken on that fillet were quite close to the surface average, while a cross indicates that at least one interior pH was significantly lower.